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Efficient L-Xylulose Production Using Whole-Cell Biocatalyst With NAD+ Regeneration System Through Co-Expression of Xylitol Dehydrogenase and NADH Oxidase in Escherichia Coli

Authors : Mesfin Angaw Tesfay

Abstract : L-Xylulose is a potentially valuable rare sugar used as starting material for antiviral and anticancer drug development in pharmaceutical industries. L-Xylulose exist in a very low concentration in nature and have to be synthesized from cheap starting materials such as xylitol through biotechnological approaches. In this study, cofactor engineering and deep eutectic solvent were applied to improve the efficiency of L-xylulose production from xylitol. A water-forming NAD+ regeneration enzyme (NADH oxidase) from Streptococcus mutans ATCC 25175 was introduced into E. coli with xylitol-4-dehydrogenase (XDH) of Pantoea ananatis resulting in recombinant cells harboring the vector pETDuet-xdh-SmNox. Further, three deep eutectic solvents (DES) including, Choline chloride/glycerol (ChCl/G), Choline chloride/urea (ChCl/U), and Choline chloride/ethylene glycol (ChCl/EG) have been employed to facilitate the conversion efficiency of L-xylulose from xylitol. The co-expression system exhibited optimal activity at a temperature of 37 ℃ and pH 8.5, and the addition of Mg2+ enhanced the catalytic activity by 1.19-fold. Co-expression of NADH oxidase with XDH enzyme resulted in increased L-xylulose concentration and productivity from xylitol as well as the intracellular NAD+ concentration. Two of the DES used (ChCl/U and ChCl/EG) show positive effects on product yield and the ChCl/G has inhibiting effects. The optimum concentration of ChCl/U was 2.5%, which increased the L-xylulose yields compared to the control without DES. In a 1 L fermenter the final concentration and productivity of L-xylulose from 50 g/L of xylitol reached 48.45 g/L, and 2.42 g/L.h respectively, which was the highest report. Overall, this study is a suitable approach for large-scale production of L-xylulose from xylitol using the engineered E. coli cell.

Keywords : Xylitol-4-dehydrogenase, NADH oxidase, L-xylulose, Xylitol, Coexpression, DESs
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